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Evaluation of traditional formulation for laxative activity in mice

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ABSTRACT

The herbal formulation of *Azadirachta indica* Juss., *Cassia angustifolia* Vahl., and padar lavan (black salt) is widely used by local traditional practitioners for constipation disorders in rural North Karnataka, India. The present study was undertaken to evaluate safety and efficacy of the formulation by employing the propulsive motility model. Herbal formulation showed significant ($P \leq 0.01$) increase in the intestinal transit time and supports for traditional use.

Keywords: *Azadirachta indica*, *Cassia angustifolia*, Laxative, Black salt

INTRODUCTION

Constipation and evacuation difficulty symptoms are common in the general population, refers to bowel movements that are infrequent or hard to pass. It is characterized by passage of lumpy or hard stools, requiring straining, sensation of incomplete evacuation, sensation of anorectal obstruction or blockage and fewer than three defecations per week [1]. The intestinal transit may be hastened by several different types of drugs, including laxatives, fecal softeners and stimulant purgatives. Laxatives generally act by enhancing retention of intra-luminal fluid by hydrophilic or osmotic mechanisms, decreasing net absorption of fluid by effects on small and large bowel fluid and electrolyte transport or altering motility by either inhibiting segmenting (non-propulsive) contractions or stimulating propulsive contractions [2, 3]. Since earliest times, medicinal plants have been of a great help to mankind bringing relief from various diseases. It has been claimed by traditional practitioners in rural Northern Karnataka that the formulation of two different medicinal plants *Azadirachta indica* Juss., *Cassia angustifolia* Vahl., with padar lavan (black salt) gives relief from constipation and patient's experiences support the claim. *Azadirachta indica* belongs to Meliaceae family, contains diterpenoids, margolone, nimbonone, nimbonolone and nimbolinin. The extracts of *Azadirachta indica* has been reported for its antibacterial, antifungal, antimalarial, antiviral, nematicidal, antifertility, spermicidal, antitumor, antiulcer, anti-inflammatory, hepatoprotective and antianxiety activities [4]. *Cassia angustifolia* belongs to Caesalpinaceae family, contains sennoside, hydroxyanthracene glycosides. These glycosides stimulate colon activity and thus have laxative action. Further, these glycosides increase fluid secretion by the colon, with the effect of softening the stool and increasing its bulk [5]. The extract of *Cassia angustifolia* exhibited antitumor, trypsin inhibitory and antifungal activities[6]. Padar lavan (Black salt) consists primarily of sodium chloride and traces of sodium sulfate, iron sulphate and hydrogen sulfide. Padar lavan is considered a cooling spice in ayurvedic medicine and is used as a laxative and digestive aid, also effective in the treatment of goiter [7, 8]. Formulation was speculated to cause diarrheal activity and accordingly, experiment was carried out on Swiss mice to explore the same. The present study was therefore undertaken to evaluate the safety and efficacy of the formulation.

MATERIAL AND METHODS

Documentation of the traditional practice:

The selected traditional practitioners were frequently visited and the details on disease, diagnosis, treatment, formulation and its ingredients, method of preparation, dosage and mode of administration were documented through interview and discussions with identified traditional practitioners.

Plant material:

The plants incorporated in the formulation i.e. *Azadirachta indica* Juss. (RMRC-0927), *Cassia angustifolia* Vahl. (RMRC-0926), were authenticated and their voucher specimens were deposited in the herbaria at Regional Medical Research Centre (ICMR), Belgaum. The parts of medicinal value from the plants were collected with the help of traditional practitioners.

Preparation of Formulation:

The formulation was prepared by following the exact procedures of traditional practitioners. *Azadirachta indica* (leaves powder 23.2g) *Cassia angustifolia* (leaves powder 46g), and 250mg padar lavan were mixed together thoroughly in a clean mortar and pestle. Human adult dose of the formulation prescribed by traditional healers was converted to animal equivalent dose as per conversion table devised by Paget & Barnes [9].

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Table 1: Distance traveled by charcoal meal in various treated animals (Percentage)

GROUP → TIME ↓	SALINE	CREMAFFIN	HERBAL FORMULATION
20 min	23.69 ± 0.34	35.65 ± 0.31*	51.05 ± 0.84*
40 min	45.31 ± 0.35	72.98 ± 0.91*	74.45 ± 0.74*
60 min	71.45 ± 0.28	100 ± 00	85.49 ± 0.47

* = P≤0.01

Table – 2: Total fecal out put in various treated groups

GROUP → TIME ↓	SALINE	CREMAFFIN	HERBAL FORMULATION
8 hr	0.17 ± 0.035	0.4 ± 0.04*	0.62 ± 0.03*
16 hr	0.57 ± 0.01	0.97 ± 0.03*	0.96 ± 0.02

* = P≤0.01

Animals:
Healthy adult, female Swiss mice weighing between 15-30g were procured from Shree Venkateshwara Traders, Bangalore, India. They were housed in the laboratory for about a week for acclimatization at room temperature (25 ± 3 ºC) with natural light & dark cycle and were fed with standard rat chow and tap water *ad libitum*. The study was approved by (IAEC), constituted as per CPCSEA Guidelines.

Drug and chemicals:
Charcoal, formalin and carboxymethyl cellulose were procured from Rankem; NaCl procured from Fisher scientific; cremaffin was procured from local pharmacist shop manufacture by Abbot India containing Liquid paraffin and milk of magnesia.

Acute toxicity studies:
Swiss mice of either sex weighing 15-20 g were used in the study. The animals were fasted over night (water *ad libitum*). A single dose of herbal formulation (2000 mg/kg BW) was administered orally next day and animals were observed for 14 days as per OECD guideline 423-2002 [10].

Propulsive gut motility:
After acclimatization period, animals were divided in three groups (n=6). All treatments were administered orally, group I received normal saline (10 ml/kg), group II received cremaffin (0.04 ml/kg) and group III received herbal formulation (200 mg/kg). Over night fasted mice (water *ad libitum*) were treated orally 60 minutes before administration of the charcoal meal (0.2 ml of a 4% suspension of charcoal in 2% carboxy methyl cellulose solution). The mice were sacrificed under halothane anesthesia after a particular time interval of 20, 40 and 60 minutes after the administration of charcoal meal. The entire intestine was removed and immersed in 5% formalin to halt peristalsis, then washed in running water and 3g weight was allowed on one end of the intestine for 1 minute to make them straight. The distance travelled by charcoal meal through the length of the intestine was measured and expressed as percent of the total distance from the pylorus to the cecum [11].

Total fecal output:
The test was performed on mice of either sex. Over night fasted mice (water *ad libitum*) were divided in three group (n=6). The first group serve as negative control received normal saline (10 ml/kg); the second group, serve as positive control received cremaffin (0.04 ml/kg) and the third group received the test formulation (200 mg/kg) in distilled water, by oral route. Immediately after dosing, the animals were separately placed in metabolic cages. After 8 hour of drug administration, the faces were collected frequently and weighed. Thereafter food and water were given to all mice fecal outputs were again weighed after of 16 hours.

Statistical analysis:
The results were expressed as Mean ± SEM and the data were analyzed by ANOVA followed by Dunnett's post hoc test. $P \leq 0.05$ was considered as significant.

RESULTS

Acute toxicity studies:
There was no mortality over a period of observation for 14 days in animals treated with a single over dose of

2000 mg/kg. There were no other signs of toxicity and LD_{50} was considered to be more than 2000 mg/kg.

Propulsive gut motility:
The herbal formulation increased the propulsion of charcoal meal through the gastrointestinal tract. Both cremaffin and test herbal formulation significantly ($P \leq 0.01$) increased the distance travelled by charcoal meal at 20 and 40 minutes, i.e. significantly reduced the intestinal transit time (Table 1).

Total fecal output:
The herbal formulation increased the total fecal output. Both cremaffin and test herbal formulation significantly ($P \leq 0.01$) increased total fecal output at the 8^{th} hour (Table 2).

The traditional herbal formulation shows significant laxative activity in mice and the activity was compared to that of cremaffin (Milk of magnesia 11.25ml, liquid paraffin 3.75ml per 15ml; emulsion) a commonly used laxative in clinical practices.

DISCUSSION

The laxative activity of herbal formulation was studied in mice. The results showed that an oral administration of the traditional formulation produced significant increase in fecal output in mice, these effects were similar to that of cremaffin. *C. angustifolia* one of the ingredients of this traditional formulation, belongs to anthraquinone group of laxatives effect. They produce giant migrating colonic contractions, induce water and electrolyte secretion. Dehydration and electrolyte imbalance are known adverse effects of anthraquinone laxatives [3]. This traditional formulation additionally contains padar lavan as one of its ingredients. Safety of the formulation could be attributed to padar lavan, which mainly contains sodium chloride, traces of sodium sulfate, sodium sulfate, iron sulfide, hydrogen sulfide [7, 8] balancing the electrolyte loss associated with strong water and electrolyte secretary action of sennoside, hydroxyanthracene glycosides of *Cassia angustifolia* [5]. *Azadirachta indica* reported to posses antibacterial, antifungal activities and that may be the reason to add it in the formulation [4]. Thus our study supports the claim of safety and efficacy of the traditional herbal formulation in the treatment of constipation.

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REFERENCES

[1] Gwee K.A. (2007) Irritable bowel syndrome and the Rome III criteria: for better or for worse?. Eur J Gastroenterol Hepatol, 19 (6):437-439.

[2] Rang H.P., Dale M.M., Ritter J.M., Moore P.K. (2003) Pharmacology. 6th ed. Chapter 25, The gastrointestinal tract. Edinburgh: Churchill Livingstone.

[3] Brunton L.L., Blumenthal D.K., Murri N., Dandan R.H., Knollmann B.C. (2006) Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. Chapter 37, Treatment of disorders of bowel motility and water flux; New York: McGraw-Hill.

[4] Kausik B., Chattopadhyay, Banerjee I., Ranajit K., Bandyopadhyay U. (2002) Biological activities and medicinal properties of Neem (*Azadirachta indica*). Current science, 82 (11): 1336-1345.

[5] Leng-Peschlow E. (1993) Sennoside-induced secretion and its relevance for the laxative effect. Pharmacology, 47 (1):14-21.

[6] Silva C.R., Monteiro M.R., Rocha H.M., Ribeiro A.F., Leitão A.C., Bezerra R.J., Pádula M. (2008) Assessment of antimutagenic and genotoxic potential of senna (*Cassia angustifolia* Vahl.) aqueous extract using in vitro assays. Toxicol In Vitro, 22(1): 212-218.

[7] Ali Z.A. (1999) Folk veterinary medicine in Moradabad District (Uttar Pradesh) India. Fitoterapia, 70 (4): 340–347.

[8] Agrawal H., Kotwal N. (2009) Foods Used as Ethno-medicine in Jammu. Ethno-Med, 3(1): 65–68.

[9] Laurence, D.R., Bacharach, A.L. (1964) Toxicity tests. Chapt. 6, Evaluation of drug activities: Pharmacometrics, Academic press, London and New York.

[10] OECD (2002) Guidelines for the testing of chemicals. Revised draft guidelines 423: Acute Oral Toxicity–Acute Toxic Class Method, Revised Document.

[11] Capasso F., Mascolo N., Autore G., Romano V. (1986) Laxative and the production of autocoids by rat colon. J Pharm Pharmacol, 38: 627-629.